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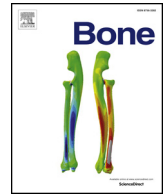
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Full Length Article

Effects of metformin, rosiglitazone and insulin on bone metabolism in patients with type 2 diabetes



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ABSTRACT

Background: Fracture risk is increased in individuals with type 2 diabetes (T2D). The pathophysiological mechanisms accentuating fracture risk in T2D are convoluted, incorporating factors such as hyperglycaemia, insulinopenia, and antidiabetic drugs. The objectives of this study were to assess whether different insulin regimens, metformin and rosiglitazone influence bone metabolism. We explored if the concentration of metformin and rosiglitazone in blood or improved glycaemic control altered bone turnover.

Methods: Two-year clinical trial designed to investigate effects of antidiabetic treatment in 371 T2D patients. Participants were randomized to short or long-acting human insulin (non-blinded) and then further randomized to metformin + placebo, rosiglitazone + placebo, metformin + rosiglitazone or placebo + placebo (blinded). Fasting bone turnover markers (BTM) representing bone resorption (CTX) and formation (PINP) including HbA1c were measured at baseline and after 3, 12 and 24 months. Trough steady-state plasma concentrations of metformin and rosiglitazone were measured after 3, 6 and 9 months of treatment. Associations between treatments and BTMs during the follow-up of the trial were analysed in mixed-effects models that included adjustments for age, gender, BMI, renal function and repeated measures of HbA1c.

Results: BTMs increased from baseline to month 12 and remained higher at month 24, with CTX and PINP increasing 28.5% and 23.0% (all: $p < 0.001$), respectively. Allocation of insulin regimens was not associated with different levels of BTMs. Metformin and metformin + rosiglitazone but not rosiglitazone alone were associated with lower bone formation (PINP). Neither metformin nor rosiglitazone plasma concentrations was associated with BTMs. HbA1c was inversely associated with CTX but not PINP.

Conclusions: The choice of insulin treatment is not influencing BTMs, metformin treatment may decrease BTMs, and improvement of glycaemic control may influence bone resorption activity.

1. Introduction

Despite higher body mass and bone mineral density (BMD) in patients with type 2 diabetes (T2D), hip fracture risk is increased by 1.4–1.7 with risks increasing to 2.7 in studies with at least ten years of follow up [1,2]. Several factors might influence fracture risk in T2D including higher prevalence of falls, renal disease, and anti-diabetic medication [3]. Insulin resistance, hyperinsulinaemia and hyperglycaemia are dominant characteristics of T2D, but limited information is available regarding their effects on bone metabolism. Most clinical

studies investigating biochemical markers of bone turnover (BTM) measured in peripheral blood or bone remodelling assessed in bone biopsies have reported lower bone turnover in T2D [4,5]. In vitro, hyperglycaemia promotes adipogenesis rather than osteogenesis, impairs osteoblast growth, increases osteoblast apoptosis [6–8] and inhibits osteoclastogenesis [9]. Insulin and insulin receptor signalling stimulate osteoblast proliferation and differentiation in human osteoblast-like cells [10], whereas insulin resistance in bone impairs bone formation and resorption in mice [11]. The sensitivity of osteoblasts and osteoclasts to insulin or hyperglycaemia from individuals with T2D

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is undetermined.

Alterations in bone metabolism due to anti-diabetic medication may influence bone metabolism both directly, e.g. by insulin promoting bone formation, and indirectly by improvement of glycaemic control [12,13]. Correction of hypoinsulinaemia in type 1 diabetes increases bone mass [14], indicating bone anabolic effects of insulin or normalisation of bone remodelling due to normoglycaemia, correction of acidosis or increased body weight, but similar investigations in patients with T2D are absent. Rather, insulin treatment is associated with increased fracture risk [13], possibly explained by an increased occurrence of hypoglycaemia, falls or renal complications. Preclinical investigations have revealed that the antidiabetic drug metformin has osteogenic effects on bone marrow progenitor cells [15], promotes osteoblast activity and reduces osteoclastogenesis [16], possibly due to activation of the AMPK and subsequently Runx2 [17], and attenuates the inhibitory effects of hyperglycaemia on osteoblast activity [18], whereas clinical studies have reported that metformin reduces or has no effect on fracture risk [13]. Pharmacogenetic investigations show that plasma metformin concentration varies considerably between individuals [19], possibly contributing to the divergence in previous results. Thiazolidinediones (TZDs) including rosiglitazone activates peroxisome proliferator-activated receptor gamma (PPARG), which stimulates the differentiation of mesenchymal stem cells into adipocytes rather than osteoblasts [20]. In effect, while improving insulin sensitivity [21], rosiglitazone increases bone resorption [12], which has been shown to increase fracture risk by 2.1 (95% CI 1.61–2.51) in women but not men [22]. The concentration of rosiglitazone differs between individuals due to genetic variations in *CYP2C8*3* [23], but it is unknown if these variations are associated with modifications in bone metabolism.

This study investigates the effects of different insulin regimens and additional treatment with metformin, rosiglitazone or placebo as well as glucose control on bone metabolism in patients with T2D. Furthermore, we explore if the concentration of rosiglitazone and metformin affect bone turnover.

2. Materials and methods

2.1. Subjects

The South Danish Diabetes Study (SDDS) has previously been described in detail [24]. In brief, SDDS was a 2-year long investigator-driven randomized controlled multicentre trial that aimed to elucidate if insulin aspart at meal times was superior to long acting NPH insulin at night in controlling glucose levels and whether the addition of metformin or rosiglitazone or both to one of the two insulin regimens improved glucose control. Candidates for SDDS were men and women with T2D who were 30–70 years old, had a BMI > 25 kg/m², a fasting C-peptide > 300 pmol/l and HbA1C > 7%. Doses used to treat T2D needed to be stable for at least 3 months. Those with congestive heart failure, impaired renal function, intolerance to metformin, or any treatment with a TZD < 30 days were excluded. Individuals treated with antiresorptive drugs such as bisphosphonates and systemic corticosteroids were excluded from all analyses.

2.2. Protocol

This study was carried out at five public hospital centres in Denmark. In all, 450 persons were included in the study, and 371 of these were eligible for the trial. The intention-to-treat population comprised 369 individuals as two were withdrawn prior to the first efficacy evaluation. After informed consent, the participants were randomly assigned to one of eight different treatment groups in a factorial design. First, all participants were randomized to either insulin aspart at mealtime or NPH insulin at bedtime. Secondly, the participants in these groups were randomly assigned to either

metformin + placebo, rosiglitazone + placebo, metformin + rosiglitazone, or placebo + placebo. Metformin, rosiglitazone and placebo treatments were blinded to the participants and investigators. Previous anti-diabetic treatment was stopped. Those assigned to insulin NPH who were insulin naïve started on 12 IU whereas participants on insulin treatment at initiation of the trial were started on 50% of their total daily dose at bedtime. Subsequently, the dose was increased until fasting blood glucose (FBG) and HbA1C were less than 5.5 mmol/l and 6.5%, respectively. Insulin naïve and participants treated with insulin at initiation of the trial who were allocated to insulin aspart were started on either 4 IU or 50% of their previous total daily dose of insulin evenly divided in three at mealtime, respectively. Doses of insulin aspart were increased until postprandial glucose levels and HbA1C were < 7.5 mmol/l and 6.5%, respectively. Insulin aspart and NPH doses were titrated rigorously for the first three months of the trial, but doses were only increased if there were no limiting cases of hypoglycaemia. Oral treatments were initiated at the start of the study. Those allocated to metformin/placebo treatment were started on 500 mg tablets twice daily, with doses being increased to 1000 mg twice daily after 4 weeks whereas those on rosiglitazone/placebo treatment started on tablets of 4 mg once daily, and the dose was increased to 8 mg once daily after 8 weeks.

Randomization was performed using a computer that generated random blocks of 8 participants. While the type of insulin was not blinded, assignment to oral treatment was double-blinded. The local ethics committee approved the trial (M-2417-02), which was registered with clinicaltrials.gov as NCT00121966.

2.3. Biochemical tests

Patients monitored capillary blood glucose daily (One Touch Ultra, LifeScan). At baseline, 3, 6, 9, 12 months and at 24 months, blood samples from the participants were drawn between 8 am and 10 am in the fasting state and stored at −80 °C until analysis. Measurements for HbA1c was included for 0, 3, 12 and 24 months. BTMs were measured in unfrozen samples collected at 0, 3, 12 and 24 months by use of a fully automated immunoassay system (iSYS, Immunodiagnostic Systems Ltd., Boldon, England) in a single run with the same batch of the reagents/assay. Serum Procollagen type I amino-terminal propeptide (PINP) and C-telopeptide of type I collagen (CTX) were measured using the chemiluminescence method. The intra- and inter-assay coefficients of variation (CV) for PINP were 3% and 5–8%, respectively (normal range in men and women 27.7–127.6 µg/l), and the intra- and inter-assay CV for CTX were < 5% and 7–10%, respectively (men 115–748 ng/l, premenopausal women 112–738 ng/l, postmenopausal women 142–1351 ng/l). The plasma concentrations of metformin and rosiglitazone were measured in unfrozen samples collected at 3, 6 and 9 months using validated high-performance liquid chromatography methods as previously described [19,25]. The lower limit of detection for metformin and rosiglitazone were 20 ng/ml and 0.25 ng/ml, and the lower limit of quantification for each drug was 30 ng/ml and 1 ng/ml, respectively [19,23]. BTMs and concentrations of medications were measured at dissimilar time points, as unfrozen samples were unavailable for measurement of BTMs at 6 and 9 months.

2.4. Statistics

A total of three patients were excluded due to concomitant intake of bisphosphonates. Basic demographics are presented as median with interquartile range (IQR). Glomerular filtration rate (eGFR) was estimated using the MDRD equation (using plasma creatinine, gender and age). Both CTX and PINP were transformed using natural logarithm to ensure a Gaussian distribution. To examine the changes in CTX and PINP we used mixed-effect modelling with restricted maximum likelihood as previously described [19,23]. Both unadjusted (not adjusted for any variables) and adjusted (adjusted for BMI and eGFR, selected a

priori) models were run. To investigate possible covariates influencing CTX and PINP concentrations, age, gender, type of insulin (aspart vs. NPH), type of oral antidiabetic drug (OAD) and HbA1c were tested in both the unadjusted and adjusted models. To assess whether metformin and rosiglitazone plasma levels correlated with CTX or PINP concentrations after three months, we performed linear regression analysis. Two models were run; one unadjusted and one model adjusted for BMI and eGFR (selected a priori). Post hoc analyses of all models were performed by including age and gender in the adjusted models. This led to no changes in the obtained conclusions. To assess the influence insulin regimen we ran a model including only BTM and insulin regimen.

The SDDS trial was designed and powered to investigate effects on glucose control, therefore, the results presented here should be considered exploratory. A two-sided *p*-value below 0.05 was considered statistically significant.

3. Results

A total of 371 patients (38% women) with a median age of 57 years (IQR: 51.5–63.0 years) were included and randomized in the South Danish Diabetes Study. Demographic data and patient characteristics at baseline, 3, 12 and 24 months are shown in Table 1. There were no differences in age, gender, body weight, and HbA1c or diabetes duration between groups [24].

The number of participants in each treatment group included: 46 on NPH insulin and placebo, 45 on NPH insulin and metformin, 46 on NPH insulin and rosiglitazone, and 46 on NPH insulin and both metformin and rosiglitazone with another 48 on insulin aspart and placebo, 45 on insulin aspart and metformin, 47 on insulin aspart and rosiglitazone and 48 on insulin aspart and both metformin and rosiglitazone [24]. During the trial, HbA1c decreased from 8.4 (7.6–9.3) to 7.1 (6.4–8.1) % (Table 1).

3.1. Changes in BTMs during the trial

The fold change in plasma levels of both CTX and PINP during the study is shown in Fig. 1, and median levels are shown in Table 1. Plasma concentration of CTX initially dropped to 78% (CI 95%: 72–84%) of the initial concentration three months after inclusion to the study, followed by a marked increase of 125% of the baseline value (CI 95%: 116–134%) 12 months after inclusion and 128% (CI 95%: 119–137%) after 24 months. Plasma concentrations of PINP were unchanged three months after inclusion, followed by 115% of the baseline value (CI 95%: 110–119%) increase 12 months after inclusion and 116% (CI 95%: 112–121%) 24 months after inclusion.

Table 1
Clinical characteristics and BTMs of 371 patients during the South Danish Diabetes Study.

	Inclusion	3 months	12 months	24 months
HbA1c (%)	8.4 (7.6–9.3)	7.6 (6.9–8.4)	7.2 (6.6–7.9)	7.1 (6.4–8.1)*
BMI (kg/m ²)	34.6 (31.4–39.3)	34.9 (31.5–39.2)	35.2 (31.8–39.6)	35.4 (31.9–39.7)
eGFR ⁺ (ml/min/1.73 m ²)	76 (67–85)	75 (66–85)	76 (67–86)	75 (65–85)
CTX (ng/l)	140 (90–230)	110 (70–180)	180 (110–290)	180 (120–300)
PINP (µg/l)	27.1 (22.0–36.7)	27.0 (21.1–34.3)	31.6 (24.7–42.9)	33.2 (25.3–43.4)

Data are shown as medians with IQR (25th–75th percentiles).

*eGFR = $186 * ((\text{serum creatinine concentration}/88.4)^{-1.154}) * (\text{age}^{-0.203}) * 0.742$ (if female).

3.2. Insulin treatment

The fold change of CTX and PINP in those treated with insulin aspart or NPH insulin during the study is shown in Fig. 2. Neither CTX nor PINP levels differed between insulin regimens (*p* = 0.38 and *p* = 0.29 in unadjusted models respectively).

3.3. CTX

In both the unadjusted and the adjusted model CTX was statistically significantly reduced at 3 months and increased at 12 and 24 months. Beta values from the full model with included covariates including repeated measures of HbA1c and corresponding confidence intervals are shown in Table 2. BMI and HbA1c were inversely correlated with CTX concentrations at 24 months while eGFR had no effect. Men had 15% (95% CI: 2–24%, *p* = 0.03) lower CTX concentrations than women in this study. Age, type of insulin or OAD regimen had no statistically significant effect on CTX concentrations. Changes in CTX after 3, 12 and 24 months according to treatment with placebo, oral antidiabetic drugs or combinations are presented in Table 2 and in Supplementary material (as Fig. 2).

3.4. PINP

In both adjusted and unadjusted analysis, PINP levels were increased at 12 and 24 months after study inclusion while no difference was observed at 3 months. Beta values from the full model with all statistically significant covariates with corresponding confidence intervals are shown in Table 2. In contrast to CTX, BMI was positively correlated with PINP at 24 months while HbA1c had no influence on PINP concentrations. Interestingly, among patients randomized to metformin alone PINP concentrations were 13% lower (CI 95%: 3–22%) while patients randomized to metformin and rosiglitazone had 21% (CI 95%: 12–29%) reduced concentrations of PINP. There was no statistically significant difference on PINP among patients randomized to metformin or patients randomized to metformin and rosiglitazone. Age, gender and type of insulin had no statistically significant effect on PINP plasma concentrations. Changes in PINP after 3, 12 and 24 months according to treatment with placebo, oral antidiabetic drugs or combinations are presented in Table 2 and in Supplementary material (as Fig. 2).

3.5. Correlations between bone turnover markers and drug concentrations

To test whether metformin or rosiglitazone could affect CTX or PINP directly, we performed regression analyses. The median plasma concentration of metformin was 628 ng/ml (IQR: 384–915 ng/ml, *n* = 148) and the median plasma concentration of rosiglitazone was 18 (IQR: 8–33 ng/ml, *n* = 176). Two models were run; one unadjusted model and one model adjusted for eGFR and BMI. The plasma concentration of metformin or rosiglitazone did not correlate with CTX or PINP (Supplementary material. Table 1).

4. Discussion

Following a minor decline in CTX but not PINP, corresponding in time to the insulin titration period, both BTMs increased in the study population and remained significantly higher by the end of the study. While allocation of insulin regimen had no effect on bone turnover, treatment with an OAD but not the plasma concentration of the OAD was associated with lower bone formation. Furthermore, as HbA1c declined during the trial and was associated with the level of the bone resorption marker, improved glycaemic control during the trial may contribute to an increase in bone turnover after 24 months.

Despite evidence of bone anabolic effects of insulin in cells and rodents, the effect of treatment with insulin on bone turnover in

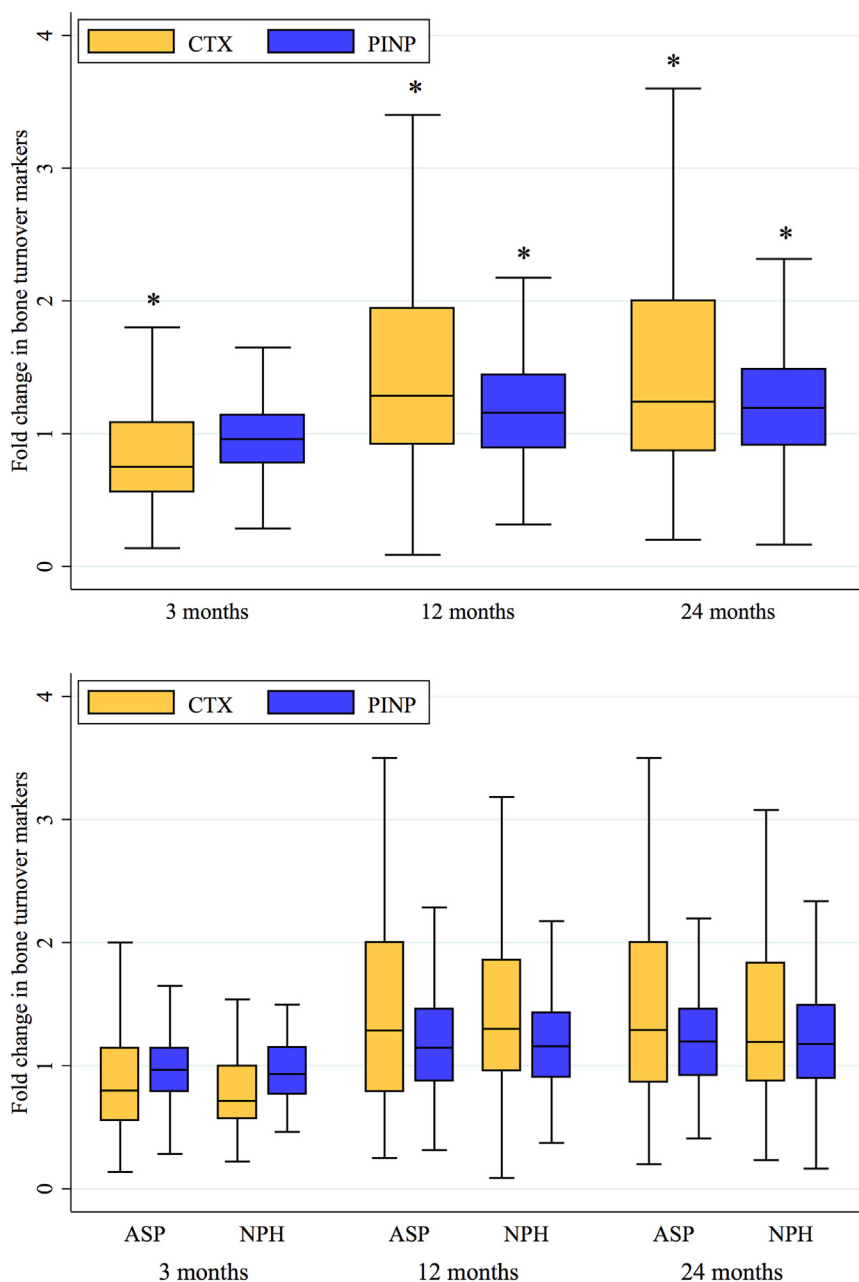


Fig. 1. Fold change in plasma levels of BTMs after 3, 12 and 24 months compared to baseline.

Fig. 1. Plasma levels of CTX decrease intermediately at three months followed by increase in CTX concentrations after 12 and 24 months. PINP was increased 12 and 24 months after inclusion.

PINP: Serum Procollagen type I amino-terminal propeptide, CTX: C-telopeptide of type I collagen. Outside values are excluded for clarity. Boxes depict median (IQR) with ranges, and those marked with a * indicate a statistically significant change compared to baseline values with a $p < 0.001$.

Fig. 2. Changes in plasma levels of BTMs after 3, 12 and 24 months compared to baseline in those treated with insulin aspart or insulin NPH.

Fig. 2. Plasma levels of CTX and PINP are similar in those treated with insulin aspart and NPH insulin after 3, 12 and 24 months.

PINP: Serum Procollagen type I amino-terminal propeptide, CTX: C-telopeptide of type I collagen. Outside values are excluded for clarity. Boxes depict median (IQR) with ranges.

individuals with T2D is unknown. At least in theory, the effect of insulin could depend on the method of exposure to the drug as in the case of parathyroid hormone (PTH), which is bone catabolic in cases of chronic hypersecretion, such as primary hyperparathyroidism, whereas exogenous administration once daily of PTH is bone anabolic [26]. However, our data showed no difference in effects of short and long acting insulin on bone metabolism. Importantly, these results do not exclude differences in fracture risk between different insulin regimens. Indeed, those on insulin aspart experienced more cases of hypoglycaemia as previously reported [24], which may increase the risk of falls and subsequently fractures [27]. There were not enough fractures in this study to provide any meaningful analysis of correlations with either drug use or BTMs.

Based on available preclinical data, metformin was expected to increase bone formation and reduce bone resorption [15,17,28]. By contrast, we observed lower level bone formation without concomitant reduction in bone resorption in those on metformin treatment, and there was no association between plasma metformin concentrations and

BTMs, not favouring a bone anabolic effect of metformin in humans with T2D. These results are partly in agreement with the ADOPT trial, a double-blinded trial designed to compare the antidiabetic effects of rosiglitazone, glimepiride and metformin in individuals with T2D, which showed lower levels of CTX and PINP after one year in those treated with metformin [29]. The ADOPT trial was designed to investigate glycaemic control using monotherapy [30]; therefore, the discrepancies in the results of the ADOPT and our trial could be explained by additional insulin therapy. In addition, reductions in Hb1Ac were numerically larger in the SDDS than the ADOPT trials, which may explain why lower levels of both BTMs were observed in the ADOPT trial [24,29]. Although comparisons of the outcomes of clinical trials and register-based studies are challenging and should be interpreted cautiously, our study are to some degree in concert with register-based studies showing either neutral or slightly beneficial effects of metformin on bone mass and fracture risk [31].

The effect of TZDs including rosiglitazone on bone metabolism has been investigated in several studies. Uncoupling of bone remodelling

Table 2

Longitudinal analyses of changes of the bone turnover markers C-telopeptide of type I collagen (CTX) and Procollagen type I amino-terminal propeptide (PINP) at 3, 12 and 24 months using mixed-effect modelling with restricted maximum likelihood (Adjusted models[§]). Overall effects of listed variables including repeated measures of HbA1c on BTM presented.

Bone turnover marker	Variable	Beta	95% CI	P
CTX	Baseline	1.00	Reference	–
	3 months	0.78	0.71–0.84	< 0.001
	12 months	1.23	1.13–1.33	< 0.001
	24 months	1.26	1.16–1.37	< 0.001
	eGFR ⁺	1.00	1.00–1.00	0.61
	BMI ⁺	0.98	0.97–0.99	0.002
	Age ⁺	1.01	1.00–1.01	0.17
	HbA1c ⁺	0.96	0.93–0.99	0.03
	Insulin aspart	1.00	Reference	–
	Insulin NPH	0.97	0.85–1.10	0.60
	Female	1.00	Reference	–
	Male	0.83	0.72–0.96	0.01
	No oral antidiabetic drug	1.00	Reference	–
	Metformin	0.91	0.76–1.10	0.33
	Metformin and rosiglitazone	0.98	0.81–1.17	0.80
	Rosiglitazone	1.08	0.90–1.30	0.41
PINP	Baseline	1.00	Reference	–
	3 months	1.00	0.96–1.05	0.90
	12 months	1.15	1.10–1.20	< 0.001
	24 months	1.16	1.11–1.22	< 0.001
	eGFR ⁺	1.00	1.00–1.00	0.07
	BMI ⁺	1.01	1.001–1.01	0.03
	Age ⁺	1.01	1.002–1.01	0.008
	HbA1c ⁺	1.00	0.98–1.02	0.91
	Insulin aspart	1.00	Reference	–
	Insulin NPH	0.94	0.88–1.02	0.12
	Female	1.00	Reference	–
	Male	0.95	0.88–1.03	0.19
	No oral antidiabetic drug	1.00	Reference	–
	Metformin	0.87	0.78–0.97	0.01
	Metformin and rosiglitazone	0.79	0.71–0.88	< 0.001
	Rosiglitazone	0.90	0.81–1.01	0.07

CTX: C-telopeptide of type I collagen, PINP Serum Procollagen type I amino-terminal propeptide, HbA1c: Haemoglobin A1c. BMI: Body mass index. [§]Models adjusted for all listed variables. ⁺All beta-values for continuous variables in this table are shown as an increase of BTM with the increase of one unit of the variable (e.g. one year or 1 kg/m²).

with increases in bone resorption but not formation was observed during treatment with rosiglitazone in a double-blinded, placebo-controlled clinical trial specifically designed to assess the effect of the investigational drug on bone metabolism in non-diabetic, post-menopausal women [32], in line with the ADOPT study which revealed increased bone resorption but not formation in women treated with rosiglitazone [29]. Additionally, compared to placebo, rosiglitazone reduced markers of bone formation in postmenopausal diabetic women [33] and formation but not resorption markers in postmenopausal non-diabetic women [34]. We did not observe independent effects of rosiglitazone on bone resorption or formation, but the combination of rosiglitazone and metformin was associated with a reduction in bone formation. These results indicate that concomitant treatment with insulin and metformin could influence the effects of rosiglitazone on bone metabolism, further supporting that rosiglitazone inhibits bone formation. The insignificant differences in the effects of rosiglitazone on PINP may also be explained by lower power in our investigation. Thus, we observed a 10% reduction in PINP in those on rosiglitazone, which is similar to previous reports of –13% in postmenopausal women over 14 weeks [34] and –4% in women and –13% in men over 12 months in the ADOPT trial [29].

Demonstration of independent effects of insulin or metformin on bone metabolism in humans including patients with T2D is challenging.

Assessment of bone remodelling by use of dynamic histomorphometry in bone biopsies collected before and after treatment with either of these drugs in single-arm trials or in comparison with placebo in patients with T2D is cumbersome and likely to be influenced by the independent effects of alterations in glucose levels on bone turnover. Uptake of metformin was recently shown not to occur in bone, but this does not preclude independent effects on bone metabolism [35]. While assessment of effects of metformin on bone remodelling is feasible in healthy subjects, enduring insulin therapy in non-diabetics for research purposes is inconceivable. Exposure to temporary increased levels of insulin during a hyperinsulinaemic, euglycaemic clamps (HEC) has no imminent effect on BTMs [36–38], whereas reductions in bone resorption markers were observed during HECs of extended duration (4 rather than 2 h) or hyperinsulinaemic, hypoglycaemic clamps (p-glucose reduced to 2.5 mmol/l) [36,38]. Taken together, current clinical data do not support clinically relevant effects on bone metabolism of insulin or metformin in individuals with T2D.

Prospective investigations of BTMs in patients with T2D are in shortage. Among 27 individuals with T2D followed for 5 years, an increase in CTX was observed in women but not men, whereas osteocalcin, a bone formation marker, remained at the same level [39]. Despite an initial drop in CTX, possibly explained by changes in medical therapy causing transient hyperglycaemia, increases in both PINP and CTX were observed in our study. As hyperglycaemia impairs both osteoblast and osteoclast activity, improved glycaemic control may have contributed to the increase in CTX. While increased bone resorption can be interpreted as an increase in fracture risk, these changes in CTX during intensified antidiabetic treatment may also reflect a normalisation of bone remodelling. The latter interpretation is supported by Danish reference intervals [40] showing that levels of BTMs were in the lower part of the normal range at initiation of the trial and within the normal range by end of the trial. Since bone formation and HbA1c were unrelated, our data indicate that improved glycaemic control primarily modifies bone resorption. Bone formation and resorption are generally coupled, and future investigations are needed to confirm if lowering of glucose levels causes alterations in bone resorption and not formation. PINP increased in our investigation, and we cannot exclude the possibility that changes in both PINP and CTX levels were influenced by factors such as improved nutrition and increased physical activity.

Importantly, SDDS was designed to investigate the effects of combinations of antidiabetic pharmaceuticals on glucose control in individuals with T2D, and the results presented here are therefore exploratory rather than primary outcomes. Furthermore, we assessed bone turnover using biochemical markers, which, although known to relate to bone remodelling [41], cannot provide insight into treatment effects on cortical and trabecular bone remodelling. The T2D skeletal phenotype has not been completely characterized [42] but is considered to be a state of low bone turnover [3]. Collection of bone biopsies in this investigation could have provided information on the skeletal phenotype and effects of antidiabetic treatments on bone resorption and formation, which may have been overlooked by measurement of BTMs. The conclusions drawn here need confirmation in trials that encompass sampling of information on bone mass and structure as well as bone remodelling. We were unable to evaluate the effects of insulin on bone turnover, because all participants were treated with insulin. Future investigations may deliver information on effects of insulin on bone remodelling in T2D, preferably including individuals with T2D with hypo- and hyperinsulinaemia. Furthermore, the liver clears PINP, indicating that liver disease such as steatosis may have increased PINP levels. Assessment of other biochemical markers of bone formation and resorption such as osteocalcin and tartrate-resistant acid phosphatase, respectively, may have provided further information on the relationship between antidiabetic treatment and bone remodelling as these markers may reflect somewhat more specific effects on osteoblasts and osteoclasts. Bone metabolism is influenced by sex steroid hormones such as testosterone, which is lower in individuals

with comorbidities commonly observed in patients with T2D, including hypertension and obesity [43]. Sex hormones were not measured in this investigation but may have influenced the effects of anti-diabetic drugs on bone metabolism.

The strengths of this study are mainly the opportunity to investigate the effects on bone turnover of different antidiabetic treatment combinations including different insulin regimens. We also measured plasma levels of both metformin and rosiglitazone, allowing us to assess if the effects were dependent on the concentration of these drugs in blood, which has not been reported before. Furthermore, all the participants were recruited within a geographically small area, seen at public hospitals, and did not have financial incitements to participate in the trial.

In conclusion, short- or long-acting human insulin treatments are not causing different levels of BTMs, and metformin treatment did not increase bone formation, in patients with fairly well controlled T2D. Additionally, rosiglitazone was not associated with increased bone resorption, possibly explained by simultaneous insulin treatment. Furthermore, our study shows that improved glycaemic control is associated with higher bone resorption, possibly portraying normalization rather than an abnormal increase in bone resorption. Further clinical trials investigating the effects of improved glycaemic control on bone remodelling including other biochemical markers of bone turnover are needed to confirm if lowering of glucose levels solely changes bone resorption and not formation.

Conflicts of interest

None.

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Study design: MMC, HBN, KB, JG, MF. Study conduct: TS, MMC, HBN, KB, JG, MF. Data collection: TS, MMC, NRJ, KB, JG. Data analysis: TS. Data interpretation: TS, MMC, MF. Drafting manuscript: TS, MF. Revising manuscript content: TS, MMC, NRJ, KB, JG, MF. Approving final version of manuscript: ALL.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bone.2018.04.004>.

References

- [1] P. Vestergaard, Discrepancies in bone mineral density and fracture risk in patients with type 1 and type 2 diabetes—a meta-analysis, *Osteoporos. Int.* 18 (4) (2007) 427–444.
- [2] M. Janghorbani, R.M. Van Dam, W.C. Willett, F.B. Hu, Systematic review of type 1 and type 2 diabetes mellitus and risk of fracture, *Am. J. Epidemiol.* 166 (5) (2007) 495–505.
- [3] N. Napoli, M. Chandran, D.D. Pierroz, B. Abrahamsen, A.V. Schwartz, S.L. Ferrari, I.O.F. Bone, G. Diabetes Working, Mechanisms of diabetes mellitus-induced bone fragility, *Nat. Rev. Endocrinol.* 13 (4) (2017) 208–219.
- [4] J. Starup-Linde, S.A. Eriksen, S. Lykkeboe, A. Handberg, P. Vestergaard, Biochemical markers of bone turnover in diabetes patients—a meta-analysis, and a methodological study on the effects of glucose on bone markers, *Osteoporos. Int.* 25 (6) (2014) 1697–1708.
- [5] J.S. Manavalan, S. Cremers, D.W. Dempster, H. Zhou, E. Dworakowski, A. Kode, S. Kousteni, M.R. Rubin, Circulating osteogenic precursor cells in type 2 diabetes mellitus, *J. Clin. Endocrinol. Metab.* 97 (9) (2012) 3240–3250.
- [6] W. Wang, X. Zhang, J. Zheng, J. Yang, High glucose stimulates adipogenic and inhibits osteogenic differentiation in MG-63 cells through cAMP/protein kinase A/extracellular signal-regulated kinase pathway, *Mol. Cell. Biochem.* 338 (1–2) (2010) 115–122.
- [7] N. Mercer, H. Ahmed, S.B. Etcheverry, G.R. Vasta, A.M. Cortizo, Regulation of advanced glycation end product (AGE) receptors and apoptosis by AGEs in osteoblast-like cells, *Mol. Cell. Biochem.* 306 (1–2) (2007) 87–94.
- [8] S. Botolin, L.R. McCabe, Chronic hyperglycemia modulates osteoblast gene expression through osmotic and non-osmotic pathways, *J. Cell. Biochem.* 99 (2) (2006) 411–424.
- [9] Y. Wittrant, Y. Gorin, K. Woodruff, D. Horn, H.E. Abboud, S. Mohan, S.L. Abboud-Werner, High d(+)glucose concentration inhibits RANKL-induced osteoclastogenesis, *Bone* 42 (6) (2008) 1122–1130.
- [10] J. Yang, X. Zhang, W. Wang, J. Liu, Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells, *Cell Biochem. Funct.* 28 (4) (2010) 334–341.
- [11] J. Wei, M. Ferron, C.J. Clarke, Y.A. Hannun, H. Jiang, W.S. Blazer, G. Karsenty, Bone-specific insulin resistance disrupts whole-body glucose homeostasis via decreased osteocalcin activation, *J. Clin. Invest.* 124 (4) (2014) 1–13.
- [12] B. Lecka-Czernik, Diabetes, bone and glucose-lowering agents: basic biology, *Diabetologia* 60 (7) (2017) 1163–1169.
- [13] A.V. Schwartz, Diabetes, bone and glucose-lowering agents: clinical outcomes, *Diabetologia* 60 (7) (2017) 1170–1179.
- [14] M.M. Campos Pastor, P.J. Lopez-Ibarra, F. Escobar-Jimenez, M.D. Serrano Pardo, A.G. Garcia-Cervigon, Intensive insulin therapy and bone mineral density in type 1 diabetes mellitus: a prospective study, *Osteoporos. Int.* 11 (5) (2000) 455–459.
- [15] M.S. Molinuevo, L. Schurman, A.D. McCarthy, A.M. Cortizo, M.J. Tolosa, M.V. Gangoiti, V. Arnol, C. Sedlinsky, Effect of metformin on bone marrow progenitor cell differentiation: in vivo and in vitro studies, *J. Bone Miner. Res.* 25 (2) (2010) 211–221.
- [16] Q.G. Mai, Z.M. Zhang, S. Xu, M. Lu, R.P. Zhou, L. Zhao, C.H. Jia, Z.H. Wen, D.D. Jin, X.C. Bai, Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats, *J. Cell. Biochem.* 112 (10) (2011) 2902–2909.
- [17] W.G. Jang, E.J. Kim, I.H. Bae, K.N. Lee, Y.D. Kim, D.K. Kim, S.H. Kim, C.H. Lee, R.T. Franceschi, H.S. Choi, J.T. Koh, Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2, *Bone* 48 (4) (2011) 885–893.
- [18] X. Shao, X. Cao, G. Song, Y. Zhao, B. Shi, Metformin rescues the MG63 osteoblasts against the effect of high glucose on proliferation, *J. Diabetes Res* 2014 (2014) 453940.
- [19] M.M. Christensen, C. Bräsch-Andersen, H. Green, F. Nielsen, P. Damkier, H. Beck-Nielsen, K. Brøsen, The pharmacogenetics of metformin and its impact on plasma metformin steady-state levels and glycosylated hemoglobin A1c, *Pharmacogenet. Genomics* 21 (12) (2011) 837–850.
- [20] A.A. Ali, R.S. Weinstein, S.A. Stewart, A.M. Parfitt, S.C. Manolagas, R.L. Jilka, Rosiglitazone causes bone loss in mice by suppressing osteoblast differentiation and bone formation, *Endocrinology* 146 (3) (2005) 1226–1235.
- [21] A.B. Mayerson, R.S. Hundal, S. Dufour, V. Lebon, D. Befroy, G.W. Cline, S. Enoksson, S.E. Inzucchi, G.I. Shulman, K.F. Petersen, The effects of rosiglitazone on insulin sensitivity, lipolysis, and hepatic and skeletal muscle triglyceride content in patients with type 2 diabetes, *Diabetes* 51 (3) (2002) 797–802.
- [22] Z.N. Zhu, Y.F. Jiang, T. Ding, Risk of fracture with thiazolidinediones: an updated meta-analysis of randomized clinical trials, *Bone* 68 (2014) 115–123.
- [23] T.B. Stage, M.M. Christensen, S. Feddersen, H. Beck-Nielsen, K. Brøsen, The role of genetic variants in CYP2C8, LPIN1, PPARGC1A and PPARG on the trough steady-state plasma concentrations of rosiglitazone and on glycosylated haemoglobin A1c in type 2 diabetes, *Pharmacogenet. Genomics* 23 (4) (2013) 219–227.
- [24] J. Gram, J.E. Henriksen, E. Grodum, H. Juhl, T.B. Hansen, C. Christiansen, K. Yderstraede, H. Gjesing, H.M. Hansen, V. Vestergaard, J. Hangaard, H. Beck-Nielsen, Pharmacological treatment of the pathogenetic defects in type 2 diabetes: the randomized multicenter South Danish diabetes study, *Diabetes Care* 34 (1) (2011) 27–33.
- [25] R.S. Pedersen, K. Brøsen, F. Nielsen, HPLC method for determination of rosiglitazone in plasma, *Chromatographia* 62 (3) (2005) 197–201.
- [26] R. Pacifici, T cells, osteoblasts, and osteocytes: interacting lineages key for the bone anabolic and catabolic activities of parathyroid hormone, *Ann. N. Y. Acad. Sci.* 1364 (2016) 11–24.
- [27] A.V. Schwartz, E. Vittinghoff, D.E. Sellmeyer, K.R. Feingold, N. de Rekeneire, E.S. Strotmeyer, R.I. Shorr, A.I. Vinik, M.C. Odden, S.W. Park, K.A. Faulkner, T.B. Harris, A. Health, S. Body Composition, Diabetes-related complications, glycemic control, and falls in older adults, *Diabetes Care* 31 (3) (2008) 391–396.
- [28] S. Bornstein, M. Moschetta, Y. Kawano, A. Sacco, D. Huynh, D. Brooks, S. Manier, H. Fairfield, C. Falank, A.M. Roccaro, K. Nagano, R. Baron, M. Bouxein, C. Vary, I.M. Ghobrial, C.J. Rosen, M.R. Reagan, Metformin affects cortical bone mass and marrow adiposity in diet-induced obesity in male mice, *Endocrinology* 158 (10) (2017) 3369–3385.
- [29] B. Zinman, S.M. Haffner, W.H. Herman, R.R. Holman, J.M. Lachin, B.G. Kravitz, G. Paul, N.P. Jones, R.P. Aftand, G. Viberti, S.E. Kahn, Effect of rosiglitazone, metformin, and glyburide on bone biomarkers in patients with type 2 diabetes, *J. Clin. Endocrinol. Metab.* 95 (1) (2010) 134–142.
- [30] S.E. Kahn, S.M. Haffner, M.A. Heise, W.H. Herman, R.R. Holman, N.P. Jones, B.G. Kravitz, J.M. Lachin, M.C. O'Neill, B. Zinman, G. Viberti, A.S. Group, Glycemic

- durability of rosiglitazone, metformin, or glyburide monotherapy, *N. Engl. J. Med.* 355 (23) (2006) 2427–2443.
- [31] P. Vestergaard, L. Rejnmark, L. Mosekilde, Relative fracture risk in patients with diabetes mellitus, and the impact of insulin and oral antidiabetic medication on relative fracture risk, *Diabetologia* 48 (7) (2005) 1292–1299.
- [32] T. Harslof, L. Wamberg, L. Møller, H. Stodkilde-Jørgensen, S. Ringgaard, S.B. Pedersen, B.L. Langdahl, Rosiglitazone decreases bone mass and bone marrow fat, *J. Clin. Endocrinol. Metab.* 96 (5) (2011) 1541–1548.
- [33] Z. Berberoglu, A. Gursoy, N. Bayraktar, A.C. Yazici, N. Bascil Tutuncu, N. Guvener Demirag, Rosiglitazone decreases serum bone-specific alkaline phosphatase activity in postmenopausal diabetic women, *J. Clin. Endocrinol. Metab.* 92 (9) (2007) 3523–3530.
- [34] A. Grey, M. Bolland, G. Gamble, D. Wattie, A. Horne, J. Davidson, I.R. Reid, The peroxisome proliferator-activated receptor-gamma agonist rosiglitazone decreases bone formation and bone mineral density in healthy postmenopausal women: a randomized, controlled trial, *J. Clin. Endocrinol. Metab.* 92 (4) (2007) 1305–1310.
- [35] L.C. Gormsen, E.I. Sundelin, J.B. Jensen, M.H. Vendelbo, S. Jakobsen, O.L. Munk, M.M. Hougaard Christensen, K. Brosen, J. Frøkiær, N. Jessen, In vivo imaging of human ¹¹C-metformin in peripheral organs: dosimetry, biodistribution, and kinetic analyses, *J. Nucl. Med.* 57 (12) (2016) 1920–1926.
- [36] K.K. Ivaska, M.K. Heliovaara, P. Ebeling, M. Bucci, V. Huovinen, H.K. Vaananen, P. Nuutila, H.A. Koistinen, The effects of acute hyperinsulinemia on bone metabolism, *Endocr Connect* 4 (3) (2015) 155–162.
- [37] R. Basu, J. Peterson, R. Rizza, S. Khosla, Effects of physiological variations in circulating insulin levels on bone turnover in humans, *J. Clin. Endocrinol. Metab.* 96 (5) (2011) 1450–1455.
- [38] J.A. Clowes, R.T. Robinson, S.R. Heller, R. Eastell, A. Blumsohn, Acute changes of bone turnover and PTH induced by insulin and glucose: euglycemic and hypoglycemic hyperinsulinemic clamp studies, *J. Clin. Endocrinol. Metab.* 87 (7) (2002) 3324–3329.
- [39] E.J. Hamilton, V. Rakic, W.A. Davis, S.A. Paul Chubb, N. Kamber, R.L. Prince, T.M. Davis, A five-year prospective study of bone mineral density in men and women with diabetes: the Fremantle diabetes study, *Acta Diabetol.* 49 (2) (2012) 153–158.
- [40] N.R. Jørgensen, L.T. Mollehave, Y.B.L. Hansen, N. Quardon, L. Lylloff, A. Linneberg, Comparison of two automated assays of BTM (CTX and P1NP) and reference intervals in a Danish population, *Osteoporos. Int.* 28 (7) (2017) 2103–2113.
- [41] P.D. Delmas, Clinical use of biochemical markers of bone remodeling in osteoporosis, *Bone* 13 (Suppl. 1) (1992) (S17–21).
- [42] R.J. Fajardo, L. Karim, V.I. Calley, M.L. Bouxsein, A review of rodent models of type 2 diabetic skeletal fragility, *J. Bone Miner. Res.* 29 (5) (2014) 1025–1040.
- [43] M. Frost, K. Wraae, T. Nielsen, D. Hougaard, K. Brixen, C. Hagen, M. Andersen, Similar reference intervals for total testosterone in healthy young and elderly men. Results from the Odense androgen study, *Clin. Endocrinol. (Oxf)* 78 (5) (2013) 743–751.